Semiconductor Properties of Melanins Prepared from Catecholamines

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Abstract. D. C. dark - and photoconductivity measurements were performed with synthetic melanins prepared by oxidative polymerization of dopamine, adrenaline, adrenochrome and adrenolutin. The melanins examined show significant differences in conductivity, thermal activation energy and photocurrent intensity values. The differences in semiconductor properties observed between the malanins reflect the structure differences of catecholamine-melanin polymers.

Key words: Model neuromelanins — Catecholamine-melanins — Semiconduction — Photoconductivity

Introduction

It is generally assumed that catecholamines are precursors of rheomelanins found in human blood and of neuromelanins localized in the cytoplasm of catecholaminergic neurons in the human brain stem and basal ganglia (Rodgers and Curzon 1975; Hegedus 1977; Graham 1978). The biological function of these melanins is not clear. It has been suggested that plasma soluble rheomelanins may represent some kind of transport forms of melanins in the body (Hegedus and Altschule 1970). The melanin contents in the human locus coeruleus and substantia nigra increase with ageing reaching a maximum at the age of about 60 years (Mann and Yates 1974; Graham 1979). On the basis of these observations it was postulated that neuromelanins are waste products of the catecholamine metabolism. On the other hand, the melanization of a particular brain structure during early childhood (Mann and Yates 1974), biosynthesis of melanin pigments in the catecholamine neurons of albino men (Foley and Baxter 1958) and decrease of pigment levels in nerve cells observed with various neurological and psychiatric disorders (Forrest 1974) suggest that neuromelanins have significant physiological functions. McGinness and Proctor (1973) postulated that neuromelanins may play a protective function acting as some

"device" for radiationless conversion of the energy of excited, and therefore potentially cytotoxic molecules, to phonons. It was also speculated that neuromelanins may participate in bioelectronic processes in pigmented neurons of the human brain through phonon-electron coupling (Lacy 1984). These suggestions have been based on the amorphous semiconductor theory used by McGinness (1972) and McGinness et al. (1974) to explain the unique electronic properties of melanin polymers such as threshold and memory switching and photoconductivity.

Semiconductive properties of melanins have been investigated mainly for tyrosine-derived natural melanins isolated from eyes, hair or melanomas and for synthetic dopa-melanin (McGinness 1972; Filatovs et al. 1976; Crippa et al. 1978; Strzelecka 1982a; Strzelecka 1982b; Jastrzębska and Wilczok 1987; Jastrzębska et al.1989). Therefore in the present study we describe some basic semiconductor characteristics of model neuromelanins obtained in vitro by oxidative polymerization of dopamine, adrenaline, adrenochrome and adrenolutin. The two latter compounds are intermediates in the oxidative pathway from adrenaline to melanin polymers (Heacock 1965).

Materials and Methods

Preparations of melanins

Melanins were obtained by oxidative polymerization of dopamine, adrenaline, adrenochrome (3-hydroxy-1-methyl-5,6-indolinedione) and adrenolutin (3,5,6-trihydroxy-1-methylindole) solutions in TRIS-HCl buffer (0.05 mol/l; pH = 7.4). The solutions of dopamine (5 mmol l) and adrenaline (5 mmol l) were aerated at 22 °C for 72 h. After acidification with concentrated hydrochloric acid to a final pH of 2.0, the precipitated melanins were separated by centrifugation (1500 g, 20 min), dialyzed against distilled water and dried P_2O_5.

In other experiments dopamine and adrenaline solutions (10 mmol l) were mixed with equal volume of buffered solution of copper sulfate (5 mmol l) and the obtained dopamine-copper and adrenaline-copper ion complexes were oxidized for 24 h. Then the reaction mixtures were acidified with hydrochloric acid to pH 1 and the precipitated melanins were separated by centrifugation, dialyzed against distilled water and dried as described above. The obtained melanins did not contain any copper ions detectable by ESR spectroscopy; they will be termed in the text copper catalyzed melanins: CC-dopamine-melanin and CC-adrenaline-melanin.

Adrenochrome was prepared by oxidation of adrenaline with silver oxide and used for melanin synthesis, as reported previously (Stępień et al. 1987b).

Adrenolutin was synthesized according to the method described by Hegedus and Altschule (1967). Adrenolutin solution (5 mmol/l) was aerated for 24 h and the reaction product was separated as described for adrenaline and dopamine.

Measurements of dark- and photoconductivity

For D.C. dark- and photoconductivity measurements melanins were dried over P_2O_5 to constant weight, powdered and pressed under the pressure of 0.1 MPa yielding tablets 1 mm thick and 5 mm in diameter. The sample was placed in a sandwich-type cell between two platinum electrodes, which
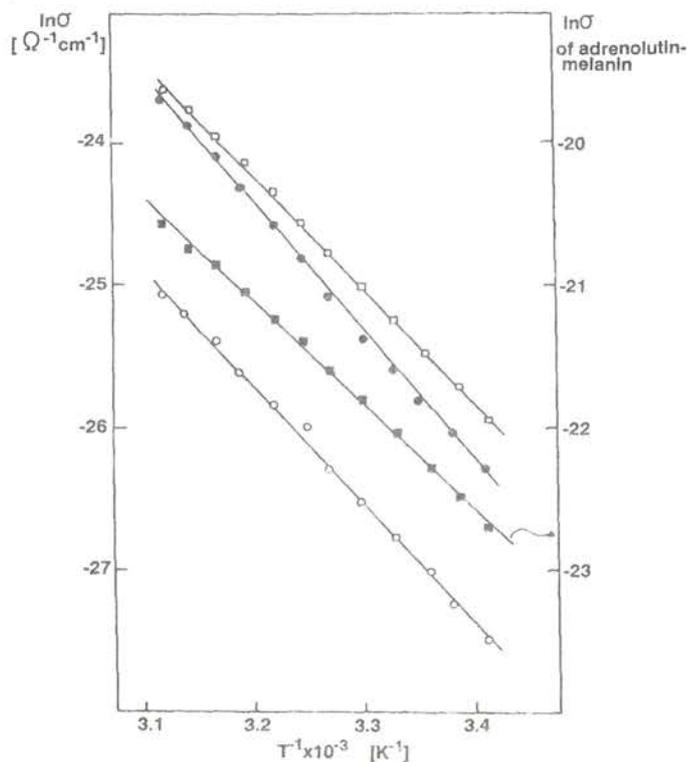
Fig. 1. The dependence of $\ln\sigma$ on T^{-1} for melanins prepared from adrenaline ($\bigcirc\bigcirc\bigcirc$), adrenochrome ($\bullet\bullet\bullet$), adrenolutin ($\blacksquare\blacksquare\blacksquare$) and dopamine ($\square\square\square$).

were in close contact with two thermal rollers as described previously (Jastrzębska et al. 1989). No changes in dark and photoconductivity were observed when a thin metal layer was put on the surface of the measured melanin sample or when measurements were carried out with Pt-electrodes applied to the sample surface under pressure. The sample temperature ranging within 290—330 K was checked by Cu-constantan thermoelements. The specific conductance of sample was calculated from the ohmic potential decrease on a 4MΩ standard resistor measured with an oscillating-capacitor electrometer "Statron" (GDR).

During photoconductivity measurements the sample was irradiated with a 450 W xenon lamp with an optical glass filter absorbing infrared radiation (Colour optical glass, CZC-26, USSR). The intensity of the applied electrical field reached 10^3Vcm^{-1}. The luminous flux was directed into the cell by means of a light pipe, whose end was equipped with a translucent platinum electrode. The light intensity at the surface of the melanin tablet was 1350 lx.
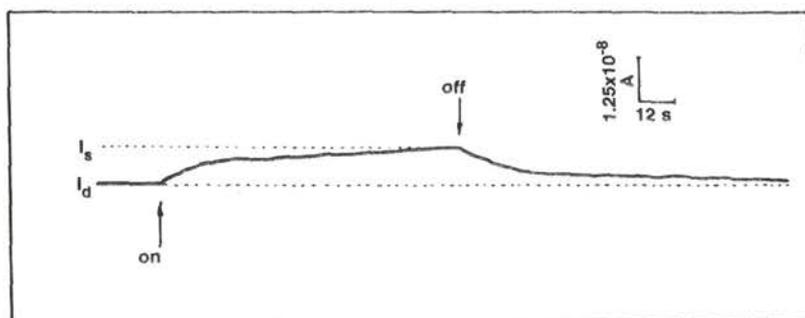
Fig. 2. Photoconductivity of adrenolutin-melanin. The rise and the decay of the photocurrent upon switching the light on and off. I_d- dark current intensity, I_s- current saturation value.

Results

Electrical dark conductivity σ measured for melanins prepared from dopamine, adrenaline, adrenochrome and adrenolutin shows a rise with the increasing temperature, according to the relation (Cohen 1971):

$$\sigma = \sigma_o \exp\left(-\Delta E_A/kT\right) \qquad (1)$$

where σ_o is the preexponential factor, ΔE_A is the thermal activation energy of conductivity, and k is the Boltzmann constant. Fig. 1 presents the dependence of $\ln\sigma$ on $(T)^{-1}$ for the melanins investigated. This dependence is linear with the slope giving the value of the thermal activation energy. Equation (1) allows also to determine the preexponential factor σ_o, which is connected with the carrier mobility. For intrinsic semiconductors, σ_o is given by formula (Meier 1974):

$$\sigma_o = e(\mu_n + \mu_p)(N_cN_v)^{1/2} \qquad (2)$$

where μ_n and μ_p are the mobilities of electron and hole respectively, N_c is the effective density of states in the conduction band, and N_v is the effective density of states in the valence band.

Fig. 1 and 3 show that the relation between $\ln\sigma$ and T^{-1} slightly differs from straight line. The deviation shows but weak significance and it can be related to water evaporation from melanin as well as to some thermally induced structure changes (Wilczok et al. 1987). The values of conductivity σ_{293} $(T = 293K)$, thermal activation energy ΔE_A and factor σ_o for the melanins analyzed are presented in Table I. Melanin obtained by oxidative polymerization of adrenolutin has the highest conductivity value and the lowest value of thermal activation energy. The conductivities of melanins from dopamine, adrenaline
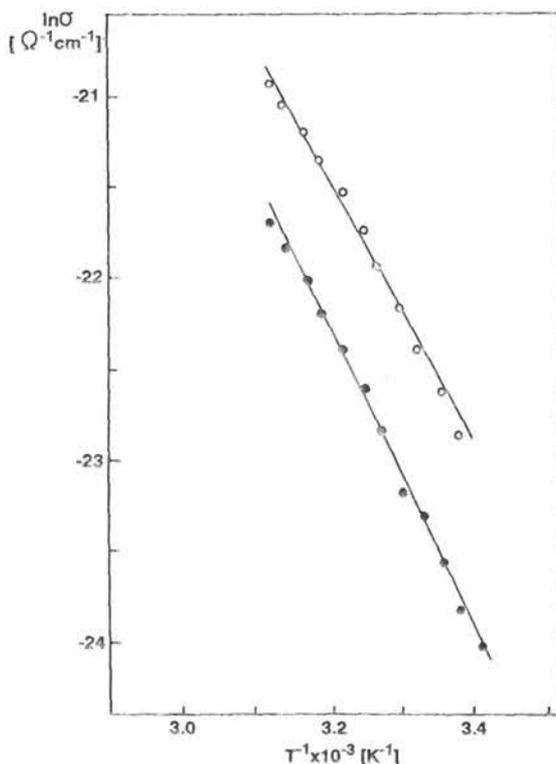
Fig. 3. The dependence of $\ln\sigma$ on T^{-1} for CC-adrenaline-melanin (O O O) and CC-dopamine-melanin (● ● ●). See "Materials and Methods" for details.

and adrenochrome are similar (the same order of magnitude), but the values of factor σ_o and of thermal activation energy are higher for dopamine- and adrenochrome-melanins than for melanin prepared from adrenaline. Illumination with visible radiation induces photoelectrical signal from adrenolutin-melanin, whereas no light-induced variations of conductivity were observed for melanins from adrenaline, adrenochrome and dopamine. Fig. 2 illustrates the rise and the decay of the photocurrent during switching the light on and off. The photocurrent increases up to a saturation value I_s and after switching the light off, it decreases approximately reaching the initial dark value within a relatively long time. The photocurrent intensity ΔI_{ph} calculated as the difference between the dark current intensity I_d and the saturation photocurrent was 8.3×10^{-9}A.

Dark- and photoconductivity measurements were made also for CC-melanins prepared from adrenaline and dopamine in the presence of copper ions,

Table I. Basic semiconductor properties of melanins prepared from catecholamines; D. C. dark- and photoconductivity measurements

	Conductivity $(T = 293K)$ σ_{293} $(\Omega^{-1}cm^{-1})$	Thermal activation energy ΔE_A (eV)	Preexponential factor σ_o $(\Omega^{-1}cm^{-1})$	Photocurrent intensity ΔI_{ph} (A)
Adrenaline-melanin	$(1.3 \pm 0.1) \times 10^{-12}$	0.68 ± 0.01	0.6	*
Adrenochrome-melanin	$(5.2 \pm 0.1) \times 10^{-12}$	0.73 ± 0.01	18.6	*
Adrenolutin-melanin	$(1.5 \pm 0.1) \times 10^{-10}$	0.62 ± 0.01	7.0	8.3×10^{-9}
Dopamine-melanin	$(5.1 \pm 0.1) \times 10^{-12}$	0.71 ± 0.01	8.0	*

* no photocurrent was observed

Table II. Basic semiconductor properties of CC-melanins prepared from adrenaline and dopamine in the presence of copper ions*

	Conductivity $(T = 293K)$ σ_{293} $(\Omega^{-1}cm^{-1})$	Thermal activation energy ΔE_A (eV)	Preexponential factor σ_o $(\Omega^{-1}cm^{-1})$	Photocurrent intensity ΔI_{ph} (A)
CC-adrenaline-melanin	$(1.3 \pm 0.1) \times 10^{-10}$	0.62 ± 0.01	6.0	5.0×10^{-9}
CC-dopamine-melanin	$(4.2 \pm 0.1) \times 10^{-11}$	0.72 ± 0.01	100.0	1.8×10^{-9}

* These melanins were treated with hydrochloric acid to remove copper ions as described in "Materials and Methods".

which were completely removed from these melanins by a treatment with dilute hydrochloric acid as described in "Materials and Methods". The results are presented in Figs. 3 and 4, and in Table II. It can be seen that melanin from adrenaline prepared in the presence of copper ions has higher values of condutivity and of factor σ_o than does melanin formed from the same precursor in the absence of Cu^{2+}. In addition, all investigated semiconductor parameters of CC-adrenaline-melanin and of adrenolutin-melanin are very similar (Tabs. I and II). Both melanins produce a photoelectrical signal and have identical values of thermal activation energy. Table II shows that CC-dopamine-melanin has higher values of σ_{293} and of factor σ_o than does melanin prepared from dopamine without copper ions. The increase of σ_o is about two orders of magnitude but the activation energies are similar. CC-dopamine-melanin shows
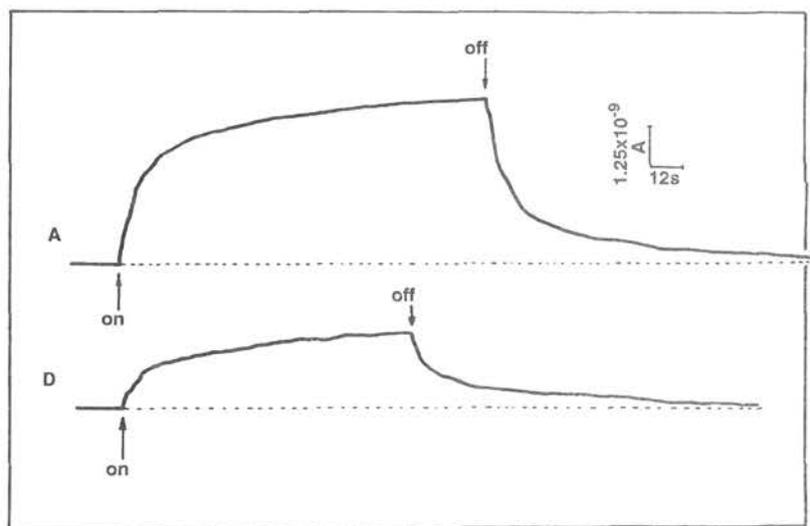
Fig. 4. Photoconductivity of CC-adrenaline-melanin (*A*) and CC-dopamine-melanin (*D*). See "Materials and Methods" for details.
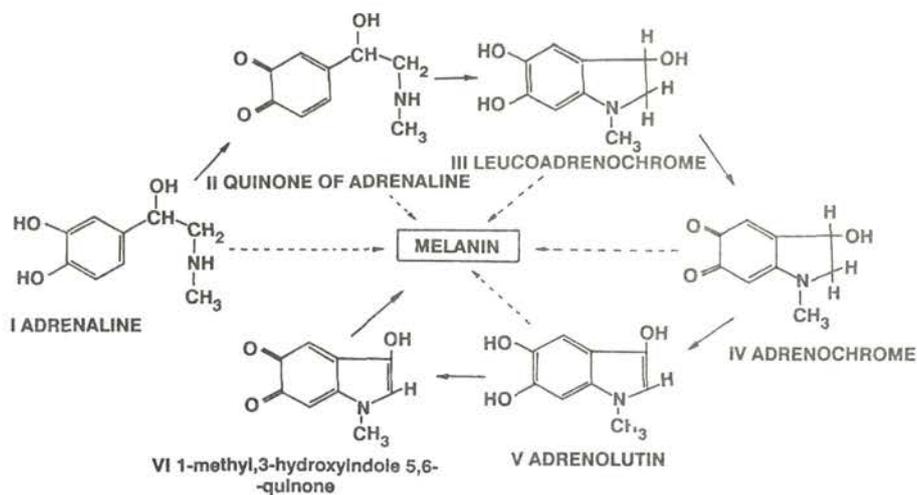
Fig. 5. Intermediate products of adrenaline conversion during melanin synthesis.

a photoelectrical signal upon illumination (Fig. 4), whereas no photocurrent was observed for dopamine-melanin.

Discussion

The electrical dark conductivity measured for melanins prepared by oxidative polymerization of catecholamines shows a temperature dependence characteristic for the activation processes, given by relation (1). This type of dependence was found earlier for other melanin biopolymers, e.g. synthetic dopa-melanin (Strzelecka 1982b), synthetic pheomelanins (Jastrzębska et al.1989), natural melanins isolated from bovine eyes and human dark hair (Strzelecka 1982a). The results reported in this paper show that the conductivities of melanins prepared from adrenaline, adrenochrome, adrenolutin and dopamine at $T = 293K$ are within the range of 1.3×10^{-12} to $1.5 \times 10^{-10} \Omega^{-1} cm^{-1}$ and at least two orders of magnitude lower than the value reported by Strzelecka (1982b) for the synthetic dopa-melanin. According to Table I the values of activation energy and of factor σ_o are higher for melanins from dopamine and adrenochrome than for adrenaline-melanin. A small rise of conductivity σ_{293} for these melanins is also observed. Assuming an unchanged effective density of states (Eq.2) we can expect a higher carrier mobility in dopamine- and adrenochrome-melanins than in adrenaline-melanin.

There are interesting differences in the semiconductor parameters between melanins from adrenaline and those from adrenolutin. As shown in Table I, the conductivity of adrenolutin-melanin is higher by about two orders of magnitude and its thermal activation energy is lower than that of adrenaline-melanin. It is known that for classes of substances of similar structure the carrier concentration and hence the conductivity increases as the activation energy decreases (Meier 1974). This is also true for melanins prepared from adrenaline and adrenolutin. The observed rise of the dark conductivity of adrenolutin-melanin is mainly connected with the higher carrier concentration in this melanin type. The higher carrier concentration is due to changes in the chemical structure of the melanin. For many organic solids a relationship has been established between the thermal activation energy of dark conductivity ΔE_A and the chemical structure of molecules: ΔE_A decreases with the increasing number of rings or π electrons (Meier 1974). For molecules with strongly delocalised π electrons the activation energy of conductivity is small. The changes observed in ΔE_A and σ_{293} of adrenolutin-melanin are due to structural differences between this type of melanin and melanin prepared from adrenaline. The process of melanin formation was shown to involve oxidative cyclization of adrenaline to adrenochrome, isomerization to adrenolutin and then polymerization to melanin (Graham 1978) (Fig. 5). The major monomer units of adrenolutin-melanin are of the indole type (the units of V and VI, Fig. 5), whereas adrenaline-melanin is a heteropolymer derived from the linkage of different monomers being transient forms of the adrenaline-melanin pathway. The high degree of homogeneity of

adrenolutin-melanin and the presence of mainly the indole units can result in an increase of the carrier concentration and thus of the conductivity. The increase of conductivity can probably be attributed also to a higher carrier mobility, because the preexponential factor σ_o is higher for adrenolutin-melanin than for melanin from adrenaline. The discussed effects were also responsible for the appearance of the photocurrent upon illumination of adrenolutin-melanin, whereas no photocurrent was observed for adrenaline- and adrenochrome-melanins.

Dark- and photoconductivity measurements were made also for melanins prepared from adrenaline and dopamine in the presence of copper ions. The results clearly indicate that the presence of copper ions during oxidative poly-merization of dopamine and adrenaline affects the semiconductor properties of the formed melanins. For CC-adrenaline-melanin and adrenaline-melanin the conductivity increases with decreasing ΔE_A. This relationship suggests an in-creased carrier concentration in melanin obtained from adrenaline in the presence of copper ions.

For CC-dopamine-melanin the increase of conductivity can probably be attributed to an enhanced carrier mobility, since the value of factor σ_o is significantly higher for this melanin than for dopamine-melanin.

The effects observed are not due to the presence of copper ions in CC-mela-nin polymers. It was found by ESR-spectroscopy and radiochemical studies that 0.1 mol/l hydrochloric acid is able to completely remove metal ions from mela-nin (Stępień et al. 1989). The different conductivities of melanins prepared in the presence and in the absence of copper ions are due to changes in the chemical structure of these melanins. Previously it was demonstrated that copper ions catalyzed nonenzymic oxidation of catecholamines to melanin (Stępień et al. 1987a). The activating effect of copper ions is associated with the formation of catecholamine-copper complexes. Recently we found that the structure of the product of the adrenaline-copper complex oxidation after removal of the fixed copper ions by treatment with dilute hydrochloric acid is similar to that of adrenolutin-melanin (Stępień et al. 1989). The electric parameters of melanin prepared from adrenaline in the presence of copper ions (Table II) are very similar to those of adrenolutin-melanin (Table I). This correspondence in semi-conductor properties supports the assumption of a similarity of the chemical structure of both melanins.

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